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13. ABSTRACT ***(Maximum 200 Words)***

Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Diets high in vegetables and fruits have been associated with reduced risk of breast cancer in many epidemiologic studies. Minor components of diet such as micronutrients including vitamins may be involved in mediating these associations, but it is not known which micronutrients are involved or how they act. Such knowledge is critical to rationally design and to implement a preventive strategy against breast cancer. Folate, a B vitamin mostly found in vegetables and fruits, may be a protective micronutrient in diet. It is a crucial component in DNA methylation as well as DNA synthesis, both of which are important processes in etiology of breast cancer. Certain genes involved in these processes differ from on person to another. Therefore, a portion of the general population with inherited sub-optimal folate metabolism along with low folate intake may be at increased risk of developing breast cancer. We plan to use a multi-disciplinary approach to study both nutritional and genetic aspects of the disease. We will investigate: (1) whether dietary folate is a micronutrient that is protective against breast cancer; (2) whether a proportion of the population with inherited sub-optimal folate metabolism is at increased risk of breast cancer; (3) whether such inherited variability modifies the association of folate intake and risk of breast cancer; and (4) whether folate may interact with alcohol that interrupts folate metabolism in contributing to risk of breast cancer.

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INTRODUCTION

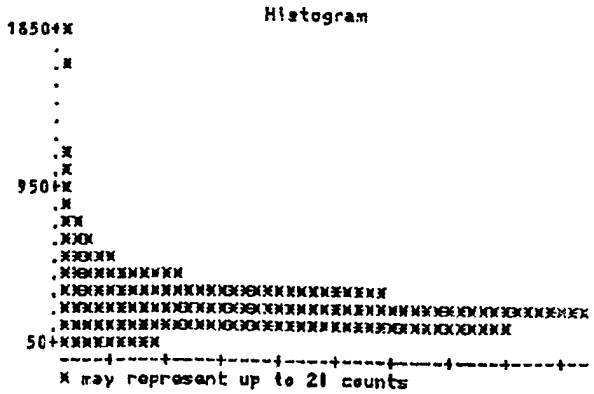
Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Low levels of folate and related B vitamins both in diet and in circulating have been associated with risk of breast cancer in several prospective epidemiologic studies. Folate, a B vitamin mostly found in vegetables and fruits, is a crucial component in DNA methylation as well as DNA synthesis, both of which are important processes in etiology of breast cancer. Certain genes involved in these processes differ from on person to another. Therefore, a portion of the general population with inherited sub-optimal folate metabolism along with low folate intake may be at increased risk of developing breast cancer. We plan to utilize resource of the Long Island Breast Cancer Project, a large population-based case-control study, to study both nutritional and genetic aspects of the disease. We will investigate: (1) whether dietary folate is a micronutrient that is protective against breast cancer; (2) whether a proportion of the population with inherited sub-optimal folate metabolism is at increased risk of breast cancer; (3) whether such inherited variability modifies the association of folate intake and risk of breast cancer; and (4) whether folate may interact with alcohol that interrupts folate metabolism in contributing to risk of breast cancer. The significance of this research lies in its potential not only to clarify etiology of breast cancer but also to guide the prevention of breast cancer through dietary modification. Furthermore, because a high proportion of the general population may inherit sub-optimal folate metabolism, attributable risk associated with these genetic factors may be quite significant. Associations between such susceptibility and risk of breast cancer will provide an extremely valuable guide to preventive dietary and other lifestyle modifications in individuals and in the population at large.

BODY

Task 1: To investigate the association of folate intake with risk of breast cancer in the Long Island Breast Cancer Study Project (Month 1-12).

a. Estimate consumption of folate and related dietary variables from food frequency questionnaire.

We have done crude analyses of dietary folate intake in the LIBCSP. Among the 3001 participants, the range of dietary intake of folate is between 0 – 1824 mg/day with mean of 270 mg/day. The distribution of dietary folate intake is illustrated below.



b. Build logistic regression models to compare breast cancer cases and population-based controls with regard to micronutrients in diet.

In order to carry out this part of the analyses, case-control status has to be unmasked. To assure unbiased genotyping effort, we decided to hold off this part of analyses still genotyping is finished to make sure researchers are blinded to case-control as well as quality control status.

Task 2: To genotype three genetic polymorphisms in folate-metabolizing genes in 1087 breast cancer cases and 1122 population-based controls of the Long Island Breast Cancer Study Project (Month 7-40).

a. Isolate and aliquot genomic DNA for genetic analysis.

DNA isolation has been completed for all 1087 cases and 1122 controls. Aliquots have been make and sent to the laboratory of Dr. Jia Chen at Mount Sinai School of Medicine, where genotyping is carried out.

b. Genotype *MTHFR* (677C->T *ala->val*; 1298A->C *glu->ala*) and *MS* (*asp*919*gly*) polymorphisms using PCR/RFLP-based methods.

We have optimized the genotyping method for *MTHFR 677C->T* and *1298A->C* polymorphisms. The methods are:

MTHFR 677C->T: The primer sequences are: 5'-TGAAGGAGAAGGTGTCTGCG GGA-3' and 5'-AGGACGGTGCGGTCAGAGTG-3'. Amplification is performed with Taq polymerase using initial denaturation at 95° C for 2 min followed by 29 cycles of 94° C for 30 s, 60° C for 30 s and 72° C for 30 s with a final extension at 72° C for 10 min. The buffer for PCR reaction include 20 mM Tris, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 7.5 mM $MgSO_4$ and 0.1% Triton X-100. The PCR products are digested with *HinfI* and size fractionated on 6% polyacrylamide gels.

MTHFR 1298A->C: The primer sequences are: 5'-GGGAGGAGCTGACCAGTGCAG-3' and 5'-GGGGTCAGGCCAGGGGCAG-3'. Amplification is performed with TaqGold polymerase using initial denaturation at 95° C for 9 min followed by 35 cycles of 94° C for 45 s, 55° C for 45 s and 72° C for 45 s with a final extension at 72° C for 5 min. The PCR products are digested with *Fnu4HI* into 119- and 19-bp fragments and size fractionated on 6% polyacrylamide gels.

We have also genotyped *MTHFR 677C->T* and *1298A-> C* polymorphisms on 442 individuals. The results are summarized below.

	677CC	*677CT*	*677TT*	*1298A->C* Total
1298AA	51	133	54	238 (54%)
1298AC	84	88	0	172 (39%)
1298CC	30	2	0	60 (14%)
677C->T Total	165 (37%)	223 (51%)	54 (12%)	442

The genotype distribution for both *MTHFR* polymorphisms are in agreement with Hardy-Weinberg Equilibrium ($p>0.7$). We also examined the linkage disequilibrium between the two *MTHFR* polymorphisms in this population. While no individuals were homozygous variant (*677TT/1298CC*) in the entire population, only 2 individuals were homozygous variant at one locus and heterozygous at the other (*677CT/1298CC*). A formal test reveals that the two polymorphisms were at linkage disequilibrium ($p<0.001$).

c. Data entry.

A master file is created containing study ID, lab ID, genotypes on all subjects.

Task 3: Final analyses and report writing (Month 36-48).

a. Analyze the associations of genetic polymorphisms in folate-metabolizing genes and risk of breast cancer.
b. Assess gene-environment interactions in relation to risk of breast cancer.
c. Investigate possible interactions of folate and alcohol consumption in ration to risk of breast cancer.
d. Prepare final report and manuscripts.

KEY RESEARCH ACCOMPLISHMENTS

1. We have performed crude analyses on the dietary folate intake in the LIBCSP.
2. We have isolated DNA from all individuals (1087 cases and 1122 controls).
3. We have genotyped 442 individuals for *MTHFR 677C->T* and *1298A->C* polymorphisms.

REPORTABLE OUTCOMES

None

CONCLUSIONS

In this first phase of the study, we have created a DNA bank for the proposed genotyping effort. We have optimized genotyping method and successfully genotyped 442 individuals on two gene loci, i.e. *MTHFR 677C->T* and *1298A->C*. The genotype frequencies for these polymorphisms are in agreement with Hardy-Weinberg Equilibrium. The two loci are also linked.

REFERENCES

None

APPENDICES

None